Remarks

I. Status of the Claims

Upon the entry of the foregoing amendment, claims 68-85 are pending in the application, with claim 68 being the independent claim. Claims 1-67 are canceled without prejudice or disclaimer of the subject matter therein. Applicants reserve rights to pursue the canceled subject matter in related applications. These changes are believed to introduce no new matter, and their entry is respectfully requested.

II. The Amendments to the Claims

Support for the amendments can be found throughout the specification as originally filed. For example, written support for the newly submitted claims can be found in the specification of the instant application as originally filed and in the specification of the parent application, *i.e.*, Application No. 10/269,473 (Atty. Dkt. No. 2147-183) ("Parent Application" hereinafter), filed October 11, 2002, as follows:

Claims	Support in the Specification as Originally Filed	Support in the Specification of the Parent Application (Appl. No. 10/269,473)
68	Paragraphs [0032], [0036], [0062], and [0083], and Figure 9	Paragraphs [0027], [0031], [0054], and [0075], and Figure 9
69	Paragraphs [0076], [0096], [0099], and [0113] - [0115]	Paragraphs [0068], [0087], [0090], and [0104] - [0106]
70-74	Paragraphs [0077], [113] - [116], and Original Claims 8-10	Paragraphs [0069], [104] - [107], and Original Claims 7-9
75-79	Paragraphs [0004], [0006], [0030],	Paragraphs [0003], [0005], [0025],

Claims	Support in the Specification as Originally Filed	Support in the Specification of the Parent Application (Appl. No. 10/269,473)
	[0037]-[0038], [0082], and [0088], and Table 2	[0032]-[0033], [0074], and [0080], and Table 2
80-81	Paragraphs [0078], [0079], and [0081], and Original Claims 15 and 18	Paragraphs [0070], [0071], and [0072], and Original Claims 13 and 16
82	Paragraphs [0011] and [0019] and Original Claim 19, 21, and 33	Paragraphs [0009] and [0016] and Original Claim 15, 17, and 24
83-85	Paragraph [0079], [0085], and [0093]	Paragraph [0071], [0077], and [0084]

Applicants respectfully request that the Examiner confirm that the the newly submitted claims are entitled to the priority date of the Parent Application. Accordingly, the amendments add no new matter, and their entry is respectfully requested.

III. Objection to the Specification

The Examiner objected to claim 9 that the specification does not have support for the term "monomeric avidin". Not agreeing with the Examiner, but solely to facilitate prosecution of this application, Applicants deleted the claim, rendering the objection moot. Applicants respectfully request that the objection be withdrawn.

IV. Objections to the Claims

The Examiner objected to claim 9 that the trademark "NEUTRAVIDIN™" is not capitalized and that a period is omitted. The Examiner also objected to claims 2, 11, 13-14, 17-18, and 26 that the terms "reconstituting" and "renaturing" are inconsistently used. Not in acquiescence of the Examiner's objections, but to expedite the prosecution of this application, Applicants deleted all claims and submitted new claims, rendering the objections moot. The newly recited claims no longer recite the trademark and the terms "reconstituting" and "renaturing." In addition, all new claims conclude with a period. Therefore, Applicants respectfully request that the objections be withdrawn.

V. Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 5 and 8 are rejected under 35 U.S.C. § 112, second paragraph, for being indefinite. In particular, the Examiner alleged that the term "low" in claim 5 is not defined in the specification. Also, the Examiner stated that the term "contacted with" in claim 8 as "it invokes a process step in the context of the instant product claims." Office Action at page 4. Applicants respectfully disagree with the assertions. However, to advance the prosecution of this application, Applicants deleted all claims and submitted new claims, rendering the rejection moot. The new claims no longer recite the terms in the rejected claims. Instead, the new claims now recite "attached to" or "binds to." Applicants submit that these terms do not limit the claims to a particular orientation of the attachment. For example, a first binding ligand attached to a monomer embraces both (1) a first binding ligand capturing and binding to a monomer and "2) a monomer capturing and binding to a first binding ligand. Furthermore, the term "attached to"

embraces any attachment or connection by one or more chemical bonds including, but not limited to, a hydrogen bond, covalent bond, ionic bond, and/or van der Waals' bond. In view of the new claims, Applicants respectfully request that the rejection be withdrawn.

VI. Rejections under 35 U.S.C. § 102(b)

Claims 1-5, 8-12, and 14-16 are rejected under 35 U.S.C. § 102 (b) as allegedly being anticipated by U.S. Patent No. 5,635,363, filed February 28, 1995 and issued June 3, 1997 ("Altman"). Not in acquiescence with the Examiner's rejection, but to advance prosecution of this application, Applicants canceled all claims and added new claims, rendering the rejection moot. Insofar as the rejection applies to the new claims, Applicants respectfully traverse.

The Examiner asserted that "Altman et al. teach a system comprising a solid surface (e.g., beads or microtiter plates) attached to a one or more MHC monomer or modified MHC monomer." Office Action at page 5. The Examiner stated that "Altman et al. teach various modified MHC monomers, including a single-chain heterodimer in which the alpha and beta subunits are fused together as a single polypeptide monomer." *Id.* The Examiner also noted that the elements of dependent claims 4, 5, 8-10, 11-12, and 14-16 are disclosed in Altman. Applicants respectfully disagree with these assertions.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference, practice, or device. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984); *see also In re Donohue*, 766 F.2d 531, 226 USPQ 619

(Fed. Cir. 1985); *PPG Industries, Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 1566 (Fed. Cir. 1996) ("[t]o anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter.").

Altman does not anticipate the newly submitted claims because Altman does not disclose or teach every element of the new claims, either expressly or inherently. The new independent claim is now directed to a system comprising a solid surface, wherein the surface is attached to a chimeric MHC class I monomer comprising a human MHC class I domain and a murine MHC class I domain, wherein said chimeric monomer maintains the ability to assemble into a ternary complex with an MHC binding peptide and beta-2 microglobulin.

Applicants respectfully submit that Altman does not disclose a system comprising a chimeric MHC class I monomer. At most, as acknowledged by the Examiner, Altman discloses a method to engineer a single molecule by fusing the α and β subunits. Office Action at page 5; *See also* Altman at col. 4, lines 22-35. In particular, Altman discloses a 'single-chain heterodimer" by fusing the two subunits using a short peptide linker such as a 15 to 25 amino acid peptide or linker. Altman at col. 4, lines 22-35. But, it fails to disclose a system comprising a solid surface attached to a chimeric MHC class I monomer of the claimed invention.

With respect to the claims depending from claim 68, Altman also fails to disclose or teach all elements of the dependent claims as the dependent claims incorporate all elements from the independent claim, *i.e.*, claim 68. Because Altman fails to disclose all elements of the independent claims, it therefore fails to disclose all elements in the

claims depending therefrom. Accordingly, in view of the newly submitted claims, Applicants respectfully argue that the new claims are not anticipated by Altman. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

VII. Rejections Under 35 U.S.C. § 103

The Examiner rejected claims 6-7 over Altman in view of U.S. Patent No. 6,485,913, filed October 2, 2000 and issued November 26, 2002 ("Becker"); claims 13 and 17-18 over Altman in view of Jager *et al.*, *J. Immunol.*, 2002, 168: 2766-2772 ("Jager") or over Altman in view of U.S. Publication No. 2003/0166057, filed December 18, 2001 and published September 4, 2003 ("Hildebrand"); claim 19 over Altman in view of Jager, or alternatively, Altman in view of Hildebrand, and further in view of Marin *et al.*, *Hybridoma*, 14(5): 443-451 (1995) ("Marin"); claims 23-24 over Altman in view of U.S. Patent No. 4,208,479, filed July 14, 1977 and issued June 17, 1980 ("Zuk"); and claims 25-26 over Altman in view of Zuk , and further in view of U.S. Patent No. 5,187,065, filed December 22, 1989 and issued February 16, 1993 ("Schutzer"). As stated above, Applicants canceled the previously pending claims and submitted new claims merely to expedite the prosecution of this application but not to acquiesce the Examiner's rejection. Insofar as the rejection applies to the new claims, Applicants respectfully traverse the rejection.

In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. *See In re Piasecki,* 745 F.2d 1468, 1471-73 (Fed. Cir. 1984). As set forth in *Graham v. John*

Deere Co. of Kansas City, "[u]nder § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject matter is determined." 383 U.S. 1, 17 (1966).

In addition, the Examiner must show reasons, explicit or otherwise, that would compel one of ordinary skill in the art to combine the references in order to make and use the claimed invention. To determine whether there is "an apparent reason to combine" the known elements in the way an application claims,

> it will be necessary. . . to look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art. . . . To facilitate review, this analysis should be made explicit.

Id. at 14; *see also* Memorandum from the United States Patent and Trademark Office, "Supreme Court decision on *KSR Int'l. Co. v. Teleflex, Inc.*," (May 3, 2007) ("The Court did not totally reject the use of 'teaching, suggestion, motivation' as a factor in the obviousness analysis. . . . [I]n formulating a rejection . . . based upon a combination of prior art elements, it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed.").

In view of the current state of the law, Applicants assert that the cited references fail to teach or suggest the claimed invention and provide no apparent reason to combine the references cited by the Examiner to arrive at the claimed invention. The new claims are now directed to a system comprising a solid surface, wherein the surface is attached to a chimeric MHC class I monomer comprising a human MHC class I domain and a

murine MHC class I domain, wherein said chimeric monomer maintains the ability to assemble into a ternary complex with an MHC binding peptide and a beta MHC subunit.

(a) Rejection over Altman in view of Becker

The Examiner rejected previously pending claims 6-7 under 35 U.S.C. § 103(a) over Altman in view of Becker. The Examiner asserted that "Altman ... teaches a system substantially as claimed." Office Action at page 7. The Examiner then noted that while Altman "fails to specifically teach that the monomer is attached reversibly or by a cleavable linkage," Becker teaches "immobilization of reagents to solid supports, in which proteins (for example) can be immobilized reversibly...." *Id.* Applicants respectfully disagree with the Examiner's assertion. However, Applicants canceled claims 6 and 7 solely to facilitate the prosecution of this application, and the newly submitted claims no longer require that the monomer be attached reversibly or by a cleavable linkage, rendering the rejection moot. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

(b) Rejection over Altman in view of Jager or Hildebrand

The Examiner rejected claims 13 and 17-18 under 35 U.S.C. §103(a) over Altman in view of Jager or Hildebrand. The Examiner stated that while Altman "fails to specifically teach that the system includes a monoclonal antibody that binds to the reconstituted, but not the denatured form of the MHC monomer," Jager teaches "methods for detecting interactions of T cells with MHC molecules, in which the monoclonal antibody w6/32 is employed in order to ensure that an equal number of

MHC/peptide complexes are used in the assay...." *Id.* at page 8. Alternatively, the Examiner asserted that "Hildebrand et al. also teach the monoclonal antibody W6/32, which binds to a conformational epitope in class I MHC molecules that includes both the heavy chain and beta2m." Office Action at page 9. As stated above, all claims have been deleted to advance prosecution of this application. Insofar as the rejection applies to the new claims, Applicants respectfully disagree.

Altman does not disclose, suggest, or otherwise contemplate the system in claim 68 or the dependent claims thereof comprising a solid surface, wherein the surface is attached to a chimeric MHC class I monomer comprising a human MHC class I domain and a murine MHC class I domain, wherein said chimeric monomer maintains the ability to assemble into a ternary complex with an MHC binding peptide and beta-2 microglobulin. As previously discussed above, Altman at most discloses a 'single-chain heterodimer' as a modified MHC monomer, which is engineered by fusing the α and β subunits using a short peptide. *See* Altman at col. 4, lines 22-35. But, it does not teach the specific chimeric MHC monomer used in the claimed invention.

The deficiencies of Altman are not cured by the disclosure in both Jager and Hildebrand. Jager does disclose a mutant HLA molecule as well as a wild-type. *See* page 2766-2767. Jager further cites Bodinier *et al.*, *Nature Medicine*, 6(6): 707-710 (2000) ("Bodinier") to indicate the specific mutant HLA molecule, noting that the mutant protein reduces nonspecific background binding of nonspecific T cells due to an altered interaction of the CD8 molecule with the HLA-A2 α3 domain. *Id.* The mutant protein described in Bodinier, attached hereto as Exhibit A, is HLA-A0201 with the substitution of valine at position 245 in the α3 domain. Exhibit A at page 708. Thus, the mutant

HLA molecule disclosed in Bodinier and cited in Jager is merely an amino acid substitution mutant not a chimeric MHC monomer comprising MHC domains from two different species, *i.e.*, human and murine. Therefore, Altman, in combination with Jager, fails to provide a system comprising a solid surface, which is attached to a chimeric MHC class I monomer. In view of the newly submitted claims and arguments above, Applicants respectfully argue that the Examiner failed to establish a *prima facie* obviousness and that the rejection be withdrawn.

Similar to Jager, Applicants respectfully note that Hildebrand fails to cure the deficiencies in Altman. Hildebrand discloses a method and apparatus for production of a soluble MHC antigen and uses thereof as well as the monoclonal antibody M6/32. *See* paragraphs [0016] - [0075]. As stated by the Examiner, Hildebrand is merely relied on as teaching an example of a monoclonal antibody, *i.e.*, W6/32. However, in view of the discussion of Altman above, Hildebrand does not render obvious Applicants' claimed system comprising a chimeric MHC monomer, which comprises a human MHC domain and murine MHC domain. Hildebrand even fails to disclose any modified or chimeric MHC monomers.

In addition to the lack of teaching or suggesting all limitations, Applicants assert that there would have been no apparent reason for one of ordinary skill in the art to combine Altman with Jager or Hildebrand. Applicants assert that the Examiner has provided no reasoning as to why one of ordinary skill in the art would adhere only a chimeric monomer comprising a human MHC domain and a murine MHC domain on a solid surface.

Therefore, Applicants respectfully argue that the Examiner failed to establish a *prima facie* case of obviousness and that this rejection should be withdrawn.

(c) Rejection over Altman in view of Jager, or alternatively, Altman in view of Hildebrand, and further in view of Marin

The Examiner rejected claim 19 for being obvious over the cited references. The Examiner asserted that "it would have been obvious to one of ordinary skill in the art to employ the B9.12.1 antibody of Marin in place of the W6/32 antibody ... in the system of Altman and Jager, or alternatively, Altman and Hildebrand." Office Action at page 11. Applicants canceled all claims and submitted new claims. To the extent that the rejection applies to the new claims, Applicants respectfully disagree with the Examiner's assertion.

As previously shown above, Altman fails to disclose, teach, or suggest all elements of the newly submitted claims. As also discussed above, Jager and Hildebrand fail to cure the deficiencies of Altman. Furthermore, Applicants respectfully submit that Marin also fails to correct the deficiencies of Altman in view of Jager, or alternatively, Altman in view of Hildebrand. Marin discloses cloning and expression of a single-chain variable fragment (ScFv) of B9.12.1 monoclonal antibody, which is specific for MHC class I molecule. *See* page 443. Marin shows that the ScFv can be used for developing new cell-typing probes and new retroviral targeting approaches. However, the combined references do not teach or suggest the claimed system comprising a solid surface, wherein the surface is attached to a chimeric MHC monomer comprising a human MHC

domain and a murine MHC domain. Similar to Jager and Hildebrand, Marin was merely used as teaching an example of the monoclonal antibody.

In addition, Applicants assert that there would have been no apparent reason for one of ordinary skill in the art to combine Altman and Jager or Hildebrand, with Marin. Applicants assert that the Examiner has provided no reasoning as to why one of ordinary skill in the art would adhere only a chimeric monomer comprising a human MHC domain and a murine MHC domain on a solid surface.

Accordingly, Applicants respectfully assert that a *prima facie* obviousness is not established and request that the rejection be withdrawn.

(d) Rejection over Altman in view of Zuk

The Examiner asserted that, with respect to claim 23, Zuk describes "that reagents for performing assays may be provided in dry form." Office Action at page 12. With respect to claim 24, the Examiner noted that Zuk teaches "that in performing assays it is a matter of substantial convenience to provide the needed reagents combined in a kit." *Id.* at page 12. Applicants respectfully disagree that the Examiner has established a *prima facie* case of obviousness of the newly submitted claims.

As previously discussed, Altman fails to disclose a system comprising a solid surface, wherein the surface is attached to a chimeric MHC class I monomer comprising a human MHC class I domain and a murine MHC class I domain. Moreover, Zuk fails to provide or suggest the disclosure lacking in Altman. Zuk merely discloses a method of detecting a substrate in an immunoassay, but fails to teach or suggest the deficiencies of Altman. *See* Abstract and Col. 2, lines 15-55.

Furthermore, there would have been no apparent reason for one of ordinary skill in the art to combine Altman with Zuk to arrive at the claimed invention. Applicants assert that the Examiner has provided no reasoning as to why one of ordinary skill in the art would adhere only a chimeric MHC class I monomer on a solid surface.

In view of the newly submitted claims and arguments above, Applicants respectfully assert that a *prima facie* case of obviousness has not been established and request that the rejection should be withdrawn.

(e) Rejection over Altman in view of Zuk, and in further view of Schutzer

The Examiner rejected claims 25-26 for being obvious over the cited references. The Examiner stated that, as shown in Schutzer, "it was well known in the art at the time of the invention to provide instructions as part of a kit for the purpose of instructing the kit user how to carry out assays with the kit." Office Action at page 20. All claims have been canceled, and new claims are submitted. Insofar as the rejection applies to the new claims, Applicants respectfully disagree.

As previously disclosed, Altman in view of Zuk fails to teach or suggest every limitation of the claims. Similar to the cited references above, Schutzer fails to provide the deficiencies of Altman and Zuk. Schutzer discloses a method of detecting the onset or presence of Lyme disease, which comprises isolating any circulating immune complexes suspected to contain antibody reactive to *Borrelia burgdorferi*. *See* Abstract. Accordingly, Altman in view of Zuk, and in further view of Shutzer fails to disclose all elements of the new claims. The Examiner merely used Schutzer as teaching an

instruction as a part of a kit not as teaching or suggesting the deficiencies in Altman and Zuk. Therefore, Applicants respectfully argue that the Examiner did not establish a *prima facie* case of obviousness and that the rejection should be withdrawn.

In addition to the lack of teaching or suggesting all limitations, Applicants assert that there would have been no apparent reason for one of ordinary skill in the art to combine Altman and Zuk with Shutzer. Applicants assert that the Examiner has provided no reasoning as to why one of ordinary skill in the art would adhere only a chimeric MHC class I monomer comprising a human MHC class I domain and a murine MHC class I domain on a solid surface.

Accordingly, Applicants assert that a *prima facie* case of obviousness with respect to the newly submitted claims has not been established. Therefore, Applicants respectfully request that the rejection under 35 U.S.C. § 103 be reconsidered and withdrawn.

Conclusion

All of the stated grounds of objections and rejections have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Date: 26 JUN 2008

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EXHIBIT A

Efficient detection and immunomagnetic sorting of specific T cells using multimers of MHC class I and peptide with reduced CD8 binding

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Tetramers of major histocompatibility complex (MHC) and peptide represent a large technological advance in immunology by allowing direct analysis of antigen-specific T cells without *in vitro* manipulation[1–4]. However, these tetramers show a concentration-dependent background binding to nonspecific T cells by interacting with the co-receptor CD8. We therefore produced tetramers of human leukocyte antigen (HLA)-A0201 and peptides, with a mutation (of alanine to valine at position 245, called V245 here) known to alter the interaction with CD8 (refs. 5 and 6). These mutated tetramers showed a greatly diminished nonspecific binding but retained specific binding, thus allowing accurate discrimination and very efficient immunomagnetic sorting of rare, specific T cells (less than 1%), without requiring the use of monoclonal antibody against CD8. Therefore, alterations of CD8 binding by mutation of the MHC greatly improved the specificity of MHC–peptide multimers, thus providing efficient tools to sort specific human T cells for immunotherapy.

Staining of T-cell clones with tetramers

We compared the binding of native and V245–HLA-A0201 (mutated) tetramers loaded with a peptide from the cytomegalovirus (CMV) pp65 protein (NLVPMVATVQ, abbreviated NLV) or the Epstein-Barr virus (EBV) BMLF1 protein (GLCTLVAML, abbreviated GLC) with those of a panel of T-cell clones of known specificities[7]. Native tetramers at a concentration of 40 μg/ml showed a substantial level of binding to irrelevant CD8⁺ T-cell clones but not to CD4⁺ T-cell clones (Fig. 1*a*), thus indicating involvement of the co-receptor CD8 in this nonspecific binding. Accordingly, addition of saturating amounts of a monoclonal antibody against CD8 decreased the binding of the native tetramer to irrelevant CD8⁺ clones (Fig. 1*a*). The comparison between HLA-A0201- and HLA-B-restricted clones indicated no correlation between nonspecific binding and HLA restriction.

Binding of mutated tetramers to specific clones was only 25–50% lower than binding of native tetramers. However, background staining on irrelevant CD8⁺ clones was abolished (Fig. 1*a*). Moreover, although nonspecific binding of native tetramers was concentration-dependent, with a large increase between 10 and 20 μg/ml, mutated tetramers did not show any background binding even at 80 μg/ml (Fig. 1*b*, A2–GLC; not shown, A2–NLV). Binding curves of native and mutated tetramers on two relevant clones showed that the native tetramer had a greater number of binding sites at the T-cell surface than the mutated tetramer (Fig. 1*c*).

These data indicate that some HLA-peptide valences of native tetramers interacted with the CD8 molecule alone on the T cell, whereas in mutated tetramers, the mutation of alanine to valine at position 245 abrogated binding of the HLA heavy chain to CD8 alone but preserved the specific interaction with T-cell antigen receptor complexes.

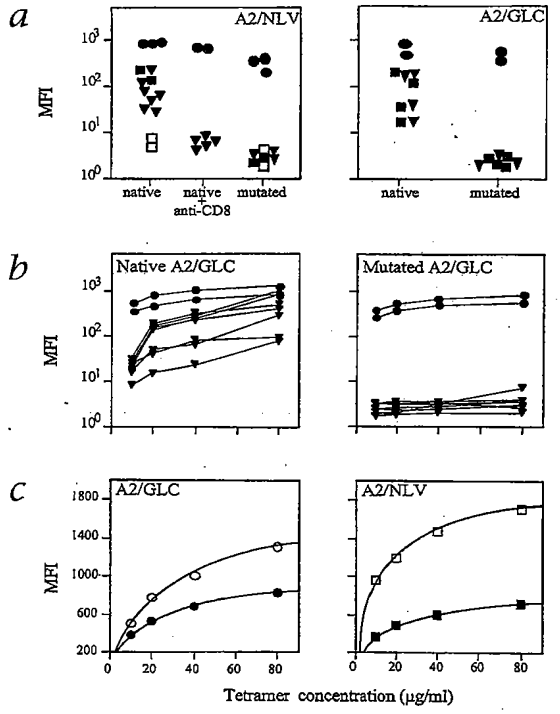


Fig. 1 Comparative labeling of T-cell clones with native and mutated HLA-A0201–peptide tetramers. ***a***. Mean fluorescence intensity (MFI) obtained after labeling GLC- or NLV-specific CD8 clones (●), irrelevant CD8 clones (HLA-A2-restricted (▼) or HLA-B-restricted (■)) or CD4 clones (□) with 40 μg/ml native or mutated tetramers loaded with NLV peptide (left) with or without 10 μg/ml monoclonal antibody against CD8 (anti-CD8) or GLC peptide (right). Specificities of irrelevant CD8 clones were IE1(CMV)/A0201, two melanoma antigens Melan-A/A0201 and Tyrosinase/A0201, BZLF1(EBV)/B4002, BZLF1/B14 and BZLF1/B18. Both CD4 clones were directed against as-yet-uncharacterized EBV peptides. ***b***, Labeling of specific (●) or irrelevant (▼) CD8 T-cell clones with increasing concentrations of native (left) or mutated (right) A2–GLC tetramers. ***c***, Specific binding curves of native (○ and □) and mutated (● and ■) tetramers on a relevant CD8 T-cell clone, A2–GLC (left) or A2–NLV (right).

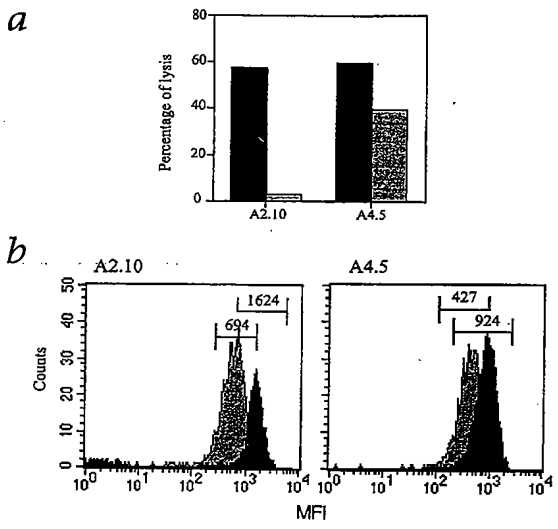
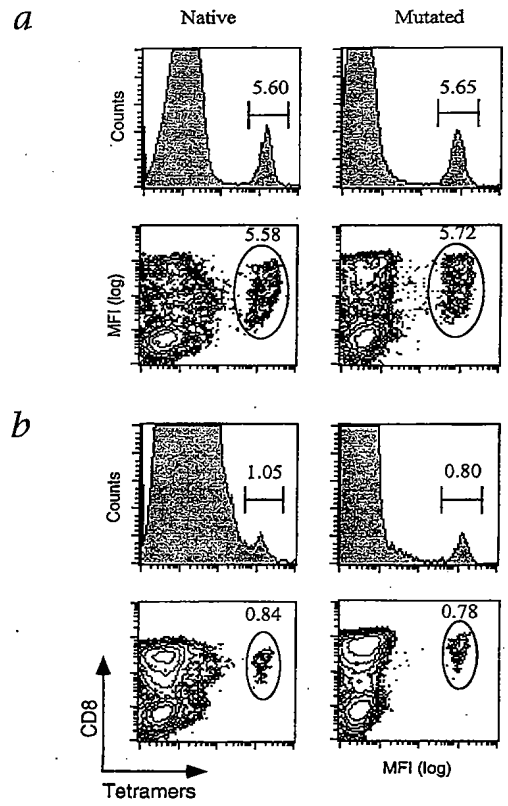


Fig. 2 Labeling of two specific T-cell clones with distinct CD8 dependencies with the mutated A2–GLC tetramer. ***a***, Cytotoxicity of both clones against GLC-pulsed HLA-A0201 target cells in the presence (■) or absence (■) of 10 μg/ml antibody against CD8. ***b***, Single staining of both clones with antibody against CD3 (grey histograms) or mutated A2–GLC tetramer at 20 μg/ml (filled histograms). Above peaks, geometric mean of fluorescence intensity (MFI). Efficiency of tetramers staining was estimated by the ratio MFI tetramer:MFI CD3. This ratio is 2.34 and 2.16 for clones A2.10 and A4.5, respectively.

Mutated tetramers bind to CD8-dependent clones

The mutation of alanine to valine at position 245 in the α3 domain of HLA-A0201 decreases its recognition by an alloreactive CD8-dependent clone but not by a CD8-independent clone[6]. We therefore tested whether V245–HLA-A0201 tetramers would bind to the T-cell receptors of CD8-dependent clones. We used two A2–GLC-specific clones, A2.10 and A4.5, which differed in their CD8 requirements for cytotoxicity, as shown by their different sensitivities to blockade by a monoclonal antibody against CD8 (Fig. 2*a*). We determined the mean fluorescence intensities after single staining with a monoclonal antibody against CD3 or the mutated A2–GLC tetramer at a concentration of 20 μg/ml (Fig. 2*b*). We estimated the efficiency of tetramer staining by the ratio of the mean fluorescence intensities of tetramer:CD3 (that is, the number of bound tetramers per number of T-cell receptors). The ratio was 2.34 and 2.16 for clones A2.10 and A4.5, respectively, indicating that dependence on CD8 did not substantially affect tetramer binding. Therefore, even though the mutation of alanine to valine at position 245 may have reduced interactions of each valence of the tetramer with the co-receptor CD8 during T-cell-receptor recognition of the CD8-dependent clone, the resulting loss of avidity of the whole tetramer was not sufficient to alter substantially its binding to the clone.



Staining of polyclonal T cells with mutated vs. native tetramers

We compared mutated and native tetramers for their accuracy to detect specific T cells within a polyclonal blood lymphocytes population after either single staining or the standard double-staining procedure using a monoclonal antibody against CD8 (refs. 1,4,8,9). We stained peripheral blood lymphocytes from two CMV$^+$ patients with rheumatoid arthritis using 20 μg/ml native or mutated A2–NLV tetramers (Fig. 3). With patient 1 (Fig. 3*a*), single staining with either of the two tetramers gave an identical estimation of the percentage of positive cells (5.6% for native and 5.65% for mutated tetramer), which was confirmed by double staining with the monoclonal antibody against CD8.

In contrast, with patient 2 (Fig. 3*b*), single staining with the native tetramer did not allow a precise determination of the percentage of positive cells because the positive peak was heavily contaminated by background staining of non-specific cells. We consistently encountered this problem with native tetramers whenever positive cells represented less than 1% of the polyclonal blood mononuclear cell population (Fig. 4, left). In these cases, addition of monoclonal antibody against CD8 allowed a precise estimation of the percentages of positive cells by decreasing background staining. This is perhaps why researchers working with native tetramer add a monoclonal antibody against CD8 to the staining reaction. Double staining showed 0.84% of positive

Fig. 3 Detection of specific T cells within a polyclonal population by single or double staining with tetramers. Peripheral blood lymphocytes from two patients with rheumatoid arthritis (***a*** and ***b***) were either single-stained (histograms) with 20 μg/ml of native (left) or mutated (right) NLV/A2 tetramers or double-stained (dot plots) with tetramers and 10 μg/ml monoclonal antibody against CD8. There is a difference in discrimination of positive cells in *b* after single staining with native or mutated tetramer. % of positive cells are indicated.

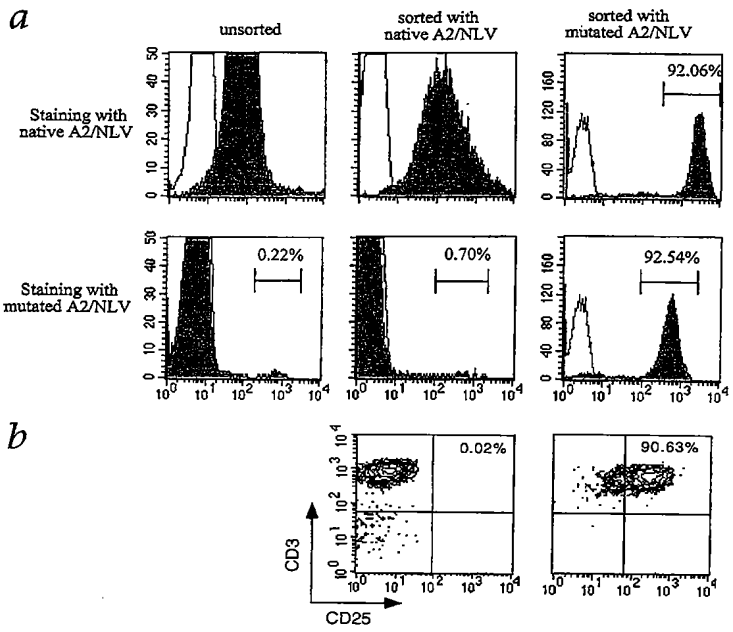


Fig. 4 Characterization of cells sorted with native or mutated tetramers. ***a***, Staining of synovial fluid T cells (95% $CD8^+$) obtained before sorting (left column), after sorting with native (middle column) or with mutated (right column) A2–NLV tetramers with 20 μg/ml of either native tetramer (top row) or mutated tetramer (bottom row). Open histograms, mutated A2–GLC tetramer (negative control). ***b***, CD25 induction in T cells sorted with native (left) or mutated (right) A2–NLV tetramers after 18 h of exposure to NLV-loaded T2 cells. % of positive cells are indicated

cells, which was very similar to results obtained after single staining with the mutated tetramer (0.8%). As expected, addition of monoclonal antibody against CD8 to the staining with mutated tetramer did not improve the discrimination of positive cells and gave identical estimations (0.8% and 0.78 for single and double staining, respectively).

These data provide further evidence that the mutation of alanine to valine at position 245 did not result in loss of reactivity towards some of the specific T cells but in fact decreased the background staining so that double staining with monoclonal antibody against CD8 was no longer required to estimate the percentage of reactive T cells accurately.

Sorting of specific T cells with mutated multimers

We initially attempted to sort specific T lymphocytes using immunomagnetic beads coated with native HLA-A0201–peptide monomers ('multimers'), as biomagnetic isolation of specific T cells using chemically biotinylated empty MHC molecules coated onto beads and loaded with peptide has been reported in the mouse[10] but not in humans. We used immunomagnetic sorting because it should prove more convenient than fluorescence-activated cell sorting to obtain clinical-grade specific T cells for immunotherapy.

Sorting efficiencies with native multimers were greatly influenced by the percentage of specific cells in the sample (that is, low percentages of specific cells resulted in poor sorting efficiencies; Table 1, SFL2, SFL6, SFL8 and PBL4). Sorted polyclonal blood mononuclear cells were 100% $CD8^+$, regardless of the percentages of specific T cells among them, thus demonstrating that CD8 interactions were essential for multimer binding.

We therefore expected to improve sorting by using mutated multimers. We sorted the same sample with native or mutated A2–NLV multimers (Fig. 4). Single staining of the initial sample with the mutated A2–NLV tetramer clearly showed a low percentage of positive cells (0.22%), whereas with the native tetramer, a small peak of positive cells within the high background staining was barely detectable.

Cells sorted with the native multimer were intermediately stained with the native tetramer, but only 0.70% of those cells were positive with the mutated tetramer. This indicated that most of these cells were irrelevant T cells whose T-cell receptor had a low affinity for the A2–NLV complex but could nevertheless bind the multimers because of CD8 interactions. To confirm the non-reactivity of the sorted cells, we expanded them *in vitro* and tested them for CD25 expression after incubation with NLV-loaded T2 cells. These sorted CD8 T cells could not be activated by NLV-loaded T2 cells (Fig. 4*b*). These irrelevant CD8 T cells were not simply 'carried over' because of insufficient washing, as we could not eliminate them in subsequent sorts (Table 1, second sort of SFL5, PBL7 and SFL8). Thus, purity could not be improved by serial sortings with native multimers.

In contrast, 92% of the cells sorted with mutated multimers were brightly stained with both native and mutated tetramers and expressed CD25 after incubation with NLV-loaded T2 cells.

Table 1 Comparative sorting efficiencies of native and mutated tetramers

Samples	Unsorted	% A2–NLV tetramer-positive cells (enrichment factor)					
		First sort with				Second sort with	
		Native A2–NLV		Mutated A2–NLV		Native A2–NLV	Mutated A2–NLV
SFL1	14.0	95.0	(6.8)				
PBL2	5.6	89.5	(16.0)	97.9	(17.5)		
SFL5	1.4	25.3	(18.1)			75.9	(3:0)
PBL3	0.8	51.1	(63.9)	97.5	(121.9)		
SFL6	0.6	13.7	(22.8)				
PBL7	nd	8.5	(nd)			12.0	(1.4)
SFL8	0.3	4.1	(13.6)			8.6	(2.1)
SFL2	0.2	0.7	(3.5)	92.5	(462.5)		
SFL4	0.14	2.9	(20.6)	97.1	(693.6)		
PBL9	0.11			75.2	(683.6)		
PBL10	0.09			82.9	(921.1)		
PBL4	0.02	0.04	(2.0)	2.2	(110.0)		98.7 (44.9)

Percentages of positive cells were assessed by staining with the native and/or the mutated A2–NLV tetramer. Samples were from HLA-A0201 rheumatoid arthritis patients or healthy CMV^+ donors (PBL9 and PBL10). nd, not determined.

Methods

Construction of the V245 mutant HLA-A0201 cDNA. Replacement of alanine 245 by valine in the α3 domain of HLA-A0201 heavy chain was accomplished using the Quick Change site-directed mutagenesis kit (Stratagene-Ozyme, Montigny-Le-Bretonneux, France). A circular PCR was done on a cDNA containing the full-length cDNA of HLA-A0201 heavy chain followed by a biotinylation sequence[1] using the following α3-modified primers: 5′–CCTTCCAGAAGTGGGTGGCTGTGGTGGT-GCC–3′ and 5′–GGCACCACCACAGCCACCCACTTCTGGAAGG–3′. The PCR product was cloned into the pHN1 expression vector and the mutation was confirmed by sequencing.

Construction of HLA-A0201–peptide tetramers. HLA-A0201–peptide monomers (native or mutated) were generated as described[11]. Recombinant proteins were produced as inclusion bodies in *Escherichia coli* XA90F LacI^q, dissolved in 8 M urea, and refolded with 15 μg/ml of pp65 peptide (NLVPMVATVQ) or BMLF1 peptide (GLCTLVAML). Tetramerization was done as described[1]. HLA monomers were biotinylated for 4 h at 30 °C with 6 μg/ml BirA (Immunotech, Marseilles, France), purified on a monoQ column (Pharmacia) and tetramerized with phycoerythrin (PE)-labeled streptavidin (Sigma) at a molar ratio 4:0.8. Tetramerization was checked on a superdex200 column (Pharmacia). There was no difference in tetramerization between native and mutated tetramers.

Cell culture. All synovial fluid lymphocytes or peripheral blood lymphocytes were obtained from HLA-A2 EBV-CMV patients with rheumatoid arthritis or from healthy HLA-A2 CMV volunteers. Viral status was assessed by serology. Specificities of lymphocyte clones were defined using overlapping peptides loaded onto the peptide transporter deficient T2 cells as described[12–14]. All clones and cell lines were maintained in RPMI medium with 10% human serum and 100 IU/ml recombinant interleukin-2 and re-stimulated every 6 weeks by polyclonal activation as described[14].

Functional assays. Cytotoxicity was measured in a standard 4-h ^{51}Cr-release assay at an effector:target ratio of 10:1 in the presence or absence of 10 μg/ml of the monoclonal antibody against CD8α, B9.11 (Coultronics SA, Margency, France) as described[14]. CD25 induction assays were accomplished by incubating tetramer-sorted cells for 18 h at 37 °C with peptide-pulsed or control T2 cells at a ratio of 1:1, before CD25/CD3 staining.

Flow cytometry. Stainings with tetramers were done at room temperature for 1 h with PE-labeled MHC-peptide tetramers and/or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against CD8 (PharMingen, San Diego, California) in PBS and 0.1% BSA. Stainings with PE-conjugated antibody against CD25 (Immunotech, Marseilles, France) and cytochrome-conjugated antibody against CD3 (PharMingen, San Diego, California) were done at 4 °C for 45 min. Stainings were analyzed by cytofluorometry.

Immunomagnetic cell sorting. Optimal conditions for coating immunomagnetic beads were determined by incubating for 1 h at room temperature HLA-A0201–peptide monomers at various concentrations (2–30 μg/ml) with 6.7×10^8 streptavidin-coated beads (Dynabeads M-280 streptavidin, DYNAL, Compiegne, France). After washes, saturation of the beads was assessed by staining with a PE-conjugated antibody against β2m (BBG6, Immunotech, Marseilles, France) and by analyzing percentages of labeled beads and mean fluorescence intensity. Saturation was achieved with a concentration of 20 μg/ml of native or mutated monomers.

Peripheral blood lymphocytes or synovial fluid lymphocytes (5×10^6–10^8; 2×10^9) were rotated for 4 h at room temperature with monomer-coated beads. After nine washes, bead-coated cells were expanded by polyclonal activation as above.

These cells were also cytotoxic against NLV-loaded HLA-A0201$^+$ target cells (data not shown) and thus were truly A2–NLV-specific.

Enrichment factors obtained with native or mutated multimers demonstrated that mutated multimers were much more efficient than native multimers in sorting specific T cells. In most cases, a single sort with mutated multimers provided highly enriched populations (more than 80%), even when specific cells represented only 0.1% of the initial sample (Table 1). Moreover, in contrast with native multimers, serial sortings with mutated tetramers could lower the threshold even further. We were able to obtain a specific population that was 98.7% pure (Table 1) and functional (data not shown) from an initial sample containing 0.02% of specific T cells.

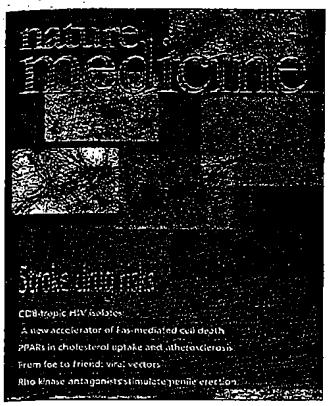
We evaluated the percentage of tetramer-positive cells captured on beads, by tetramer staining of the unbound population. After sorting PBL3, PBL9 and PBL10 with mutated multimers, the ratios obtained using the equation (% tetramer-positive unsorted—% tetramer-positive unbound)/% tetramer-positive unsorted were 97.8%, 89.5% and 87.5%, respectively, demonstrating that this procedure allowed recovery of most of the positive cells.

In conclusion, our data have shown that multimers made with mutated HLA–peptide monomers that do not bind CD8 alone are very efficient tools to detect and sort rare CD8$^+$-specific T cells and should prove extremely useful both in fundamental and therapeutic applications.

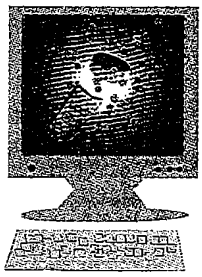
Acknowledgments

We thank A. McMichael (MRC Human Immunology Unit, Oxford) for plasmids pHN1/β2m and pHN1/HLA-A2. M.B. is supported by a fellowship from the Ligue Nationale Contre le Cancer.

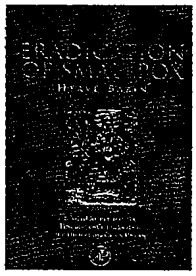
1. Altman, J.D. *et al.* Phenotypic analysis of antigen-specific T lymphocytes. *Science* **274**, 94–96 (1996).
2. Busch, D.H., Pilip, I.M., Vijh, S. & Pamer, E.G. Coordinate regulation of complex T-cell populations responding to bacterial infection. *Immunity* **8**, 353–362 (1998).
3. Bousso, P. *et al.* Individual variations in the murine T-cell response to a specific peptide reflect variability in naive repertoires. *Immunity* **9**, 169–178 (1998).
4. Lee, P.P. *et al.* Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nature Med.* **5**, 677–685 (1999).
5. Salter, R.D. *et al.* Polymorphism in the alpha 3 domain of HLA-A molecules affects binding to CD8. *Nature* **338**, 345–347 (1989).
6. Salter, R.D. *et al.* A binding site for the T-cell coreceptor CD8 on the α3 domain of HLA-A2. *Nature* **345**, 41–46 (1990).
7. Scotet, E. *et al.* T-cell response to Epstein-Barr virus transactivators in chronic rheumatoid arthritis. *J. Exp. Med.* **184**, 1791–1800 (1996).
8. Callan, M.F. *et al.* Direct visualization of antigen-specific CD8$^+$ T cells during the primary immune response to Epstein-Barr virus *in vivo*. *J. Exp. Med.* **187**, 1395–1402 (1998).
9. Ogg, G.S. *et al.* Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103–2106 (1998).
10. Luxembourg, A.T. *et al.* Biomagnetic isolation of antigen-specific CD8$^+$ T cells usable in immunotherapy. *Nature Biotechnol.* **16**, 281–285 (1998).
11. Garboczi, D.N., Hung, D.T. & Wiley, D.C. HLA-A2-peptide complexes: Refolding and crystallization of molecules expressed in *Escherichia coli* and complexed with single antigenic peptides. *Proc. Natl. Acad. Sci. USA* **89**, 3429–3433 (1992).
12. Coulie, P.G. *et al.* A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.* **180**, 35–42 (1994).
13. Gervois, N., Guilloux, Y., Diez, E. & Jotereau, F. Suboptimal activation of melanoma infiltrating lymphocytes (TIL) due to low avidity of TCR/MHC-tumor peptide interactions. *J. Exp. Med.* **183**, 2403–2407 (1996).
14. Couedel, C. *et al.* Selection and long-term persistence of reactive CTL clones during an EBV chronic response are determined by avidity, CD8 variable contribution compensating for differences in TCR affinities. *J. Immunol.* **162**, 6351–6358 (1999).



Stroke is commonly caused by brain artery obstruction, and the clot-busting blood protease tissue plasminogen activator (tPA) is used as a stroke therapy. However, tPA has also been shown to have direct effects on neurons. On page 59 of this issue, Nicole *et al.* report that proteolytic activity of tpa affects NMDA receptor-mediated signaling and promotes neuronal death. The cover panels show cultures of cortical neurons undergoing cell death in response to tPA.



Control of online medicines (page 6)



nature medicine

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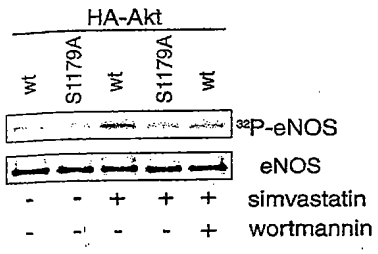
ERRATA

The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals.
YASUKO KUREISHI, ZHENGYU LUO, ICHIRO SHIOJIMA, ANN BIALIK, DAVID FULTON, DAVID J. LEFER, WILLIAM C. SESSA & KENNETH WALSH

Nature Med. 6, 1004-1010 (2000).

On page 1006, the labeling for Fig. 3*c* was incorrect. The correct labeling for Fig. 3*c* is presented here.



We regret this error.

Efficient detection and immunomagnetic sorting of specific T cells using MHC class I/peptide multimers with reduced CD8 binding.
MARIE BODINIER, MARIE-ALIX PEYRAT, CLAIRE TOURNAY, FRANCOIS DAVODEAU, FRANCOIS ROMAGNE, MARC BONNEVILLE & FRANCOIS LANG

Nature Med. 6, 707-710 (2000).

The pp65 epitope was described as the decamer NLVPMVATVQ when in fact the nonamer NLVPMVATV was used to make the tetramer and test the sorted populations.

We regret this error.

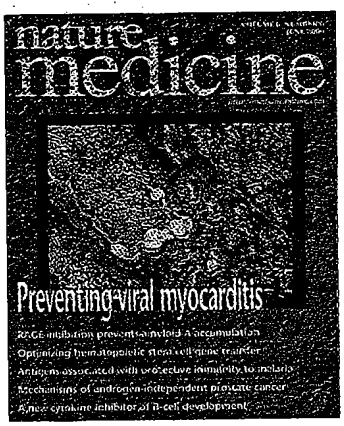
CORRECTION

Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial.
LUDWIG KAPPOS, GIANCARLO COMI, HILLEL PANITCH, JOEL OGER, JACK ANTEL, PAUL CONLON, LAWRENCE STEINMAN & THE ATLERED PEPTIDE LIGAND IN RELAPSING MS STUDY GROUP*

Nature Med. 6, 1176-1182 (2000)

The following individuals were inadvertently excluded from the list of authors in the Altered Peptide Ligand in Relapsing MS Study Group

CHRISTIAN CONFAVREUX, SANDRINE BLANC & JEAN-CLAUDE FROMENT
Hopital Neurologique, Service de Neurologie A, BP Lyon Montchat, 69394 LYON Cedex 3.



The coxsackievirus B serogroup (CB) may be a causative agent in the development of human myocarditis and cardiovascular disease, and mice infected with CB3 develop autoimmune myocarditis. On page 393, Horwitz *et al.* show that CB3 infection of transgenic mice expressing IFN-γ in their pancreatic β cells results in reduced viral replication and prevents chronic autoimmune myocarditis. The cover shows that transgenic expression of IFN-γ in the pancreas activates resident macrophages (brown staining) to defend neighboring pancreatic acinar cells after viral infection.

nature medicine

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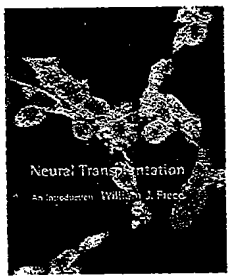
More women in trials (page 612)



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